Journal of Applied Biology & Biotechnology Vol. 11(2), pp. 253-258, Mar-Apr, 2023
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2023.110228

Development of colloidal Gold nanoparticle based lateral-flow assay for rapid detection of SARS-CoV-2 showing enhanced sensitivity and specificity

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ARTICLE INFO

Article history:
Received on: August 03, 2022
Accepted on: November 05, 2022
Available online: January 22, 2023

Key words:
Gold nanoparticle,
Monoclonal antibody,
COVID-19,
SARS-CoV-2,
Lateral flow assay.

ABSTRACT

To stop the spread of COVID-19 in this outbreak, diagnostic testing is essential. Quick diagnostic tests must be employed in this pandemic, which is brought on by the severe acute respiratory syndrome (SARS)-CoV-2 virus, to successfully treat and manage COVID-19. There are several problems with the present RT-PCR system that the lateral flow assay (LFA), a kind of clinically sensitive diagnostic test, may be able to fix, especially in low- and middle-income nations. Gold nanoparticle- (AuNP-LFA) is a practical method for detecting COVID-19 in basic hospitals and laboratories, particularly in emergency situations where many samples must be quickly examined. Safe, accurate, and non-toxic diagnostic tests must be employed during the pandemic, to successfully treat and manage COVID-19. Recombinant SARS-CoV-2 nucleocapsid monoclonal antibody was employed to detect COVID-19 antigens in the presence of patients to establish a fast LFA for COVID-19. Synthesis of colloidal gold particles and antibody colloidal gold conjugates was evaluated by using UV/Vis spectroscopy. A capture line made of anti-SARS-CoV-2 antibody was coated on nitrocellulose membrane. To create the control line, goat anti-mouse IgG monoclonal antibody was coated. On a polystyrene backing board, the immunochromatographic strip was constructed in the ideal order. Using ELISA as the standard procedure, the strips' sensitivity and specificity were assessed. The results' stability and repeatability were evaluated over a 9-month period. Colloidal gold nanoparticle-based LFAs created in this study can be employed for quicker and more accurate detection of SARS-CoV-2.

1. INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus is the infectious disease known as coronavirus disease 2019 (COVID-19). The SARS, the Middle East respiratory syndrome and the common cold are all diseases that can be brought on by the coronavirus family of viruses. A novel coronavirus was found to be the source of the sickness outbreak that occurred in China in 2019 [1]. It is known to cause COVID-19. The World Health Organization (WHO) labeled the COVID-19 outbreak a pandemic in March 2020. The most typical symptoms of COVID-19 are respiratory and it can mimic the flu, the common cold or pneumonia. In addition to other organs, COVID-19 can harm the respiratory system including the lungs [2]. The illness may also have an impact on other bodily components. A quick antigen test to identify COVID-19 infection is the Rapid COVID-19 Antigen Test (RAT), also known as the COVID-19 cross-flow test or LFT [3]. These have considerable cost advantages, can be put into place fast with little training and give customers findings in 5–15 min for a fraction of the price of other COVID-19 testing methods. In certain nations, population testing or population-wide screening methods include RAT. The nucleocapsid protein antigen of the SARS-CoV-2 virus is predominantly detected in nasal swabs or other similar clinical specimens by the COVID-19 antigen test, which is intended for the quick identification of active infections [4]. Sputum and nasal swabs were most consistently able to detect SARS-CoV-2 in the first 14 days following symptom onset, but throat swabs were unreliable 8 days after the onset of symptoms [5,6] A negative test result from respiratory sample does not exclude the condition due to the diversity in virus loads. These negative tests could be the consequence of poor sampling methods, a low viral burden in the studied area or viral genome changes [7,8].

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The SARS-CoV-2 virus in this instance has a spike protein on its surface. The tentative guidance from WHO outlining the potential benefits supported the initial scientific basis for the prospective benefits of quick diagnostics and the global direction of the development of rapid diagnostic systems. The report claims that quick testing is more cost-effective and considerably simpler to implement. The WHO advised using them to track disease trends and find cases early in an outbreak [9]. The European Commission later extended its suggestion following a spate of quickly expanding investigations. Rapid testing methods have been suggested by the European Commission for population-wide screening where the percentage of positive tests is extremely high. The Commission has committed to strengthen its stance by promoting greater use of rapid testing by January 2021, where studies show that RAT may be carried out by the testing itself [10].

Self-tests may be taken into consideration with or without professional advice. The two-site, recombinant, antibody-based fast COVID antigen test kit uses immunochromatography. It comprises of a pad covered in recombinant COVID antibodies and colloidal gold particles. The same COVID antibody is placed on a membrane in the test strip's second section. During the test, colored particles will bind to the antibody on them if COVID antigens are present in the sample. Recombinant antibodies immobilized there then follow the antibody-antigen complex through the membrane and seize it. We have created a quick test kit for SARS-CoV-2 detection that may be used in the field without a lab or specialized tools. Technical information has been published elsewhere. Here, we will introduce the process used to create and develop this test kit as an illustration of a product design exercise. Chemical product design approaches have advanced significantly in recent years, which is particularly helpful for the development of products for customers.

2. MATERIALS AND METHODS

2.1. Materials and Reagents

We bought goat anti-mouse IgG antibody, mouse IgG and SARS-CoV-2 Recombinant Nucleocapsid Monoclonal Antibody from Fapon Biotech Co., Ltd (China). Axiva Sichem Biotech in India provided the nitrocellulose (NC) membrane, absorbent pad, sample pad, conjugate pad, and PVC sheets. Sigma-Aldrich provided the hydrogen tetrachloroaurate hydrate, while Merck provided the trisodium citrate (Millipore). In our laboratory, phosphate buffer saline (PBS, pH 7.5, 0.01M in 0.85% NaCl) was created. The other compounds used in this study were all of the finest quality.

The Molecular Quest Healthcare Pvt. Ltd., Gurugram, Haryana, provided the antigen samples for the COVID-19 patients and healthy individuals. The analytically pure other reagents can be obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). An experiment's ultrapure water was made using a Millipore Milli-Q water purification system (Billerica. MA, USA). UV Spectrophotometer 1900i UV, Shimadzu, Japan, was used to gauge the colloidal gold's optical density. The BIO-DOT Dispenser from China was utilized to coat the SARS-CoV-2 antibody on the NC membrane.

2.2. Synthesis and Characterization of Colloidal Gold Nanoparticles

All glassware that was utilized, including beakers and flasks, was cleaned with ultrapure water before being dried and processed in aqua regia. According to a previously disclosed procedure, reduction of gold chloride with 1% sodium citrate resulted in colloidal gold nanoparticles with a mean particle diameter of 40 nm [11,12]. In a nutshell, 100 ml of ultrapure water were heated to boiling using electric heating in an Erlenmeyer flask with a magnetic stirrer, at which point 1 ml of a solution containing 1% gold chloride and 10 ml of a solution containing 1% sodium citrate were swiftly added to the mixture while it was being stirred. The solution was heated for another 10 min until it turned dark red, at which point it was stopped, agitated for another 15 min, cooled, and then stored at 2–8°C. UV/Vis spectroscopy was used to detect the emergence of colloidal gold at a wavelength between 530 and 535 nm [13].

2.3. Optimization and Formation of Antibody-Colloidal Gold Conjugates

The reaction conditions, which included the concentration of colloidal gold and the SARS-CoV-2 Recombinant Nucleocapsid Monoclonal Antibody, were optimized before creating the gold conjugate. With 0.2 M of K_2CO_3, the colloidal gold's pH was tuned to be 7.5 ± 0.2.50 ml of nucleocapsid recombinant monoclonal antibody were combined with the previously stated colloidal gold at concentrations of 10, 20, 30, 40, 50, 60, and 70 ng/ml, respectively. The mixture was combined with the antibody, and then let to sit at room temperature (RT) for 20 min. After incubation, 0.2 ml of 10% bovine serum albumin was added, and the mixture was then incubated for an additional 20 min at RT. After incubation, the solution was centrifuged at 10,000 rpm for 20 min at 4°C, and the supernatant was discarded. The pellet was resuspended in 0.5 ml of 20 mM borate buffer (pH 8.0), which also contained 0.5% trehalose, 0.5% bovine serum albumin, and 0.1% Tweenty-20. The mixture was then filtered through a 0.22 μm membrane. UV/Vis spectroscopy was used to track the development of the antibody-colloidal gold conjugation at a wavelength between 540 and 545 nm. For later use, the preparation was kept in storage at 4°C.

In addition, the concentration of colloidal gold was evaluated using several dilutions (1:1, 1:2, 1:3, 1:4, 1:5, and 1:6) with the ideal antibody concentration. On a piece of glass fiber membrane, the conjugated gold nanoparticle was equally sprayed or dipped and it was then dried at 45°C for 3–4 h. The test line (T-line) was made by micro-spraying COVID-19 Nucleocapsid Recombinant Monoclonal Antibody at a concentration of 2 mg/mL into 0.2 μM NC membrane using BIODOT. A similar procedure was utilized to create the control line (C-line), using goat anti-mouse IgG antibody at a concentration of 2 mg/mL that was micro sprayed onto the same NC membrane. The test membrane was dried for the entire night at 37°C and kept at 25°C. In addition, several doses of 1–2.5 mg/mL of the COVID-19 Nucleocapsid Recombinant Monoclonal Antibody and goat anti-mouse IgG antibody were optimized [13,14].

2.4. Preparation of Immunochromatographic Strip

The NC membrane was set up with a nucleocapsid recombinant monoclonal antibody coupled with colloidal gold at one end, the T-line micro-sprayed with goat anti-mouse IgG antibody upstream, and the C-line placed downstream, with a distance of 0.5 cm between the T-line and the C-line [15]. In addition, a cushioning pad was placed at the opposite end. A 2.8 mm broad by 6 cm long strip was then cut from the polystyrene backing board [Figure 1a]. After that, each strip was put into a plastic cassette and kept separately in a desiccated plastic bag. The number and placement of the detection lines on the test strips were used to determine the test results. In contrast to negative samples,

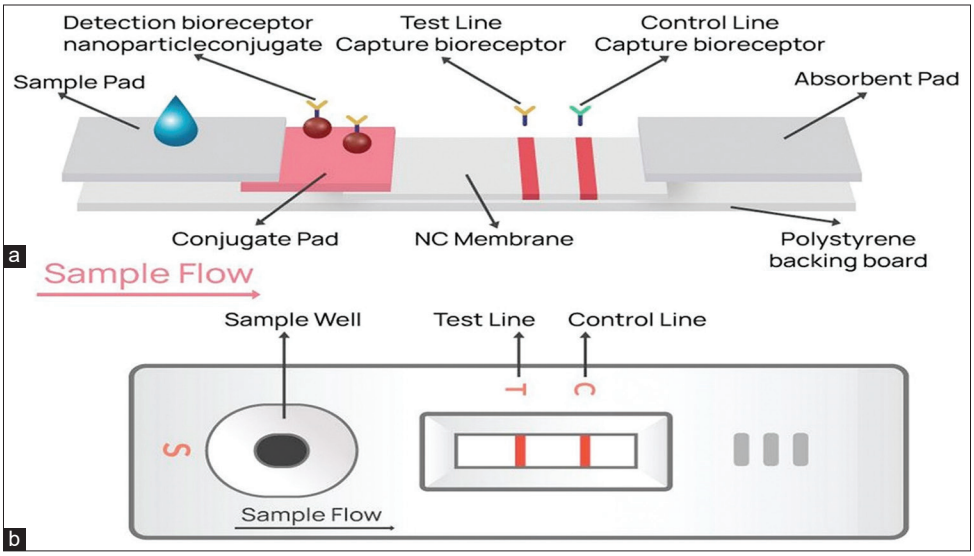

Figure 1: Nitrocellulose (NC) membrane was arranged with nucleocapsid recombinant monoclonal antibody conjugated with colloidal gold, T-line and C-line (a), test results were determined by the number and position of the detection lines on the test strips (b).

which produced one band at the C-line and no bands at the T-line, positive samples produced two bands: One at the C-line and one at the T-line. Whenever there was no band at the C-line, the findings were disregarded [Figure 1b].

2.5. Procedure of Nasopharyngeal Specimen Detection with Extraction Buffer

The SARS-CoV-2 Nucleocapsid Recombinant Monoclonal Antibody Strip's sample port was filled with 50 µL of the extraction buffer (pH 9.2) for detection, which was then added at the desired ratio of 1:100. Through capillary action, the liquid then moved toward the absorbent pad. The test cassette is made up of two parts: A colored conjugate pad with anti-SARS-CoV-2 antibodies that have been combined with colloidal gold (antibody conjugates), and a NC membrane strip with a T line and a C line. The C line is pre-coated with goat anti-mouse IgG antibody, whereas the T line is pre-coated with antibodies that are specific to SARS-CoV-2.

A swab is used to capture the specimen, and an extraction buffer is used to separate the SARS-CoV-2 antigen from the swab. After coming into touch with the test strip, the antigen extracts move across it by capillary action. If the extract contains SARS-CoV-2 antigen, it will bind to the antibody conjugates. The pre-coated anti-SARS-CoV-2 antibody then captures the immune complex on the membrane, resulting in the formation of a colorful T line and a positive COVID-19 test result. Internal control (C line) in the test should display a colored line regardless of color development on the T line [16]. The test is invalid and the specimen must be retested using a new device if the C line does not develop.

2.6. Sensitivity, Specificity and Stability of the Immunochromatographic Strip

Utilizing 100 known COVID-19 positive samples and 100 known COVID-19 negative samples, the test strips' sensitivity and specificity were assessed. The colloidal gold test strip's repeatability and stability were also estimated. The test strips were tested for repeatability and stability at intervals of 0, 1, 3, and 6 months after being stored for up to 9 months at 4°C and at RT.

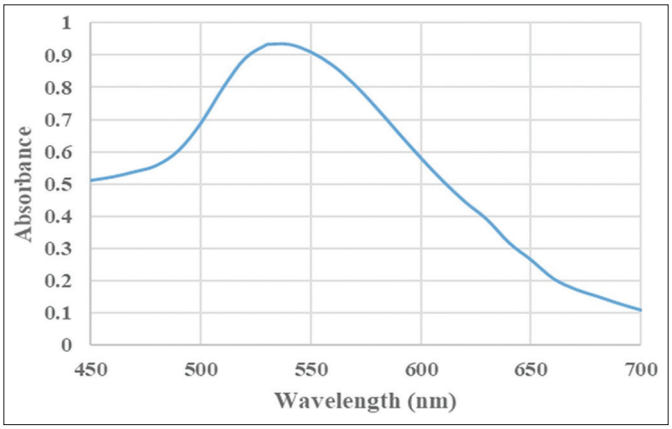

Figure 2: UV-Vis absorption spectrum of AuNPs.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of Colloidal Gold Nanoparticles

The UV-Vis absorption spectra of AuNPs are shown in Figure 2. At 534.50 nm, AuNPs showed their strongest ultraviolet absorption peak, which was consistent with the previously reported theoretical equation [17,18]. The UV-Vis absorption spectra's modest peak shape indicates that the generated AuNPs have good distribution homogeneity in the aqueous solution. The spectra of gold nanoparticles produced by the described method were measured between 650 and 450 nm in the visible spectrum with water as a standard.

3.2. Optimization and Formation of Antibody-Colloidal Gold Conjugates

The ideal antibody concentration (10–70 ng/ml) with colloidal gold was evaluated to optimize the colloid gold test strip assay. The conjugate was stable and stably captured colloidal gold particles using a minimum of 20 ng/ml SARS-CoV-2 Recombinant Nucleocapsid Monoclonal Antibody [Figure 3] [19]. The best dilution for the concentration of colloidal gold was found to be 1:2. [Figure 4a and b]. At 2 mg/mL, the performance of both coating antibodies such as

Table 1: Stability of the immunochromatographic strip.

Test Interval Month	0 Day				1 Month				3 Month				6 Month				9 Month			
Panel	2–8°C		RT		2–8°C		RT		2-8°C		RT		2–8°C		RT		2–8°C		RT	
	CL	TL	CL	TL	CL	TL	CL	TL	CL	TL	CL	TL	CL	TL	CL	TL	CL	TL	CL	TL
Strong Positive	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Medium Positive	3+	2+	3+	2+	3+	2+	3+	2+	3+	2+	3+	2+	3+	2+	3+	2+	3+	2+	3+	2+
Low Positive	3+	1+	3+	1+	3+	1+	3+	1+	3+	1+	3+	1+	3+	1+	3+	1+	3+	1+	3+	1+

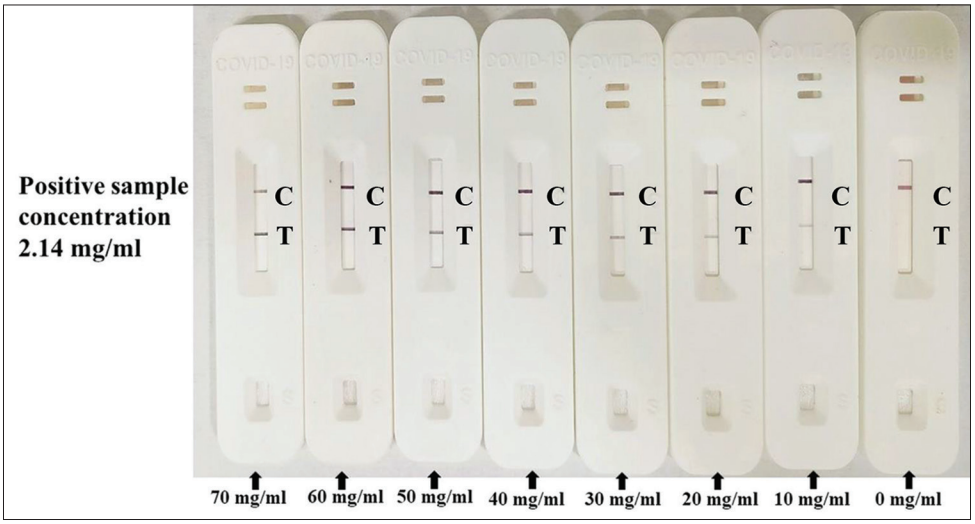

Figure 3: Figure showing the result for the different antibody concentration at fixed gold colloid concentration.

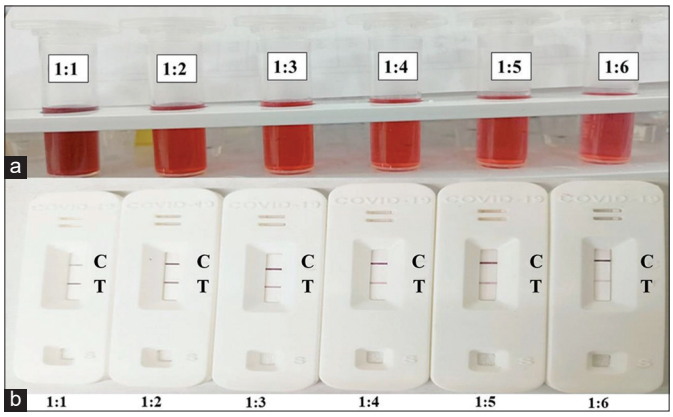

Figure 4: Figure showing the results for the fixed antibody concentration (a) and at different gold colloid concentration (b).

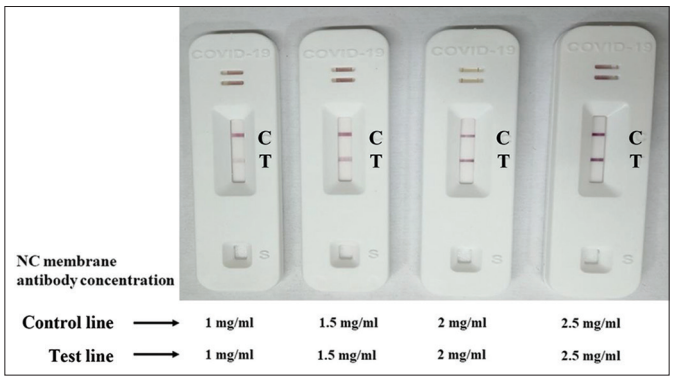

Figure 5: AuNP-LF performance based on nitrocellulose membrane coating.

goat anti-mouse IgG and COVID-19 Nucleocapsid Recombinant Monoclonal Antibody was at its best [Figure 5]. A number of parameters and the reaction conditions was adjusted to increase sensitivity and accuracy [20].

3.3. Stability of the Immunochromatographic Strip

Test strips of colloidal gold from the same batch were examined after 9 months of storage at RT and 2–8°C to determine the stability. The outcomes are displayed in Table 1. Three panels such as strong positive, medium positive, and low positive were used. Strong positive panel does not show any change for both control and T line. Whereas, medium positive panel show change of only one point between C-line and T line, and low positive panel shows change of two points between C-line and T line. All of these three panels were stable for up to 9 months. The colloidal gold test strips can be kept in storage for up to 9-months, according to stability data. A considerable portion of SARS-CoV-2 cases have ended in death, and the virus' quick global dissemination may make human infection more likely. A pandemic and the transmission of the SARS-CoV-2 virus can be avoided by quickly identifying and isolating COVID-19 patients.

In extreme situations, such as distant locations or regions with antiquated equipment or technology, the test strip can also be used as a diagnostic tool to quickly control epidemics [21-23]. The gold standard is that molecular detection methods like RT-PCR are not compatible with the sensitivity of colloidal gold immunochromatographic strips for the fast detection of SARS-CoV-2 [24]. The disadvantages of using the lateral flow assay (LFA) in comparison to the RT-PCR method are the problem with viral load levels in nasopharyngeal material and no possibility to enhance the response by enzyme reaction. Moreover, LFA will not be able to detect the amplicons.

4. CONCLUSION

This study developed colloidal gold nanoparticle-based LFA which can be utilized to detect SARS-CoV-2 quickly and accurately. Successful management and detection of COVID-19 disease is the outcome of this research. However, here in this study, the COVID-19 disease cannot be detected without the use of NC membrane, goat anti-mouse IgG antibody and absorbent pad. Glass fiber membrane, UV/Vis spectroscopy and immunochromatographic strips are few prerequisites for the result.

5. AUTHORS CONTRIBUTION

Concept and design were developed by Suresh C. Singh, Tapan K. Singh and Rajat Maheshwari. Data acquisition was done by Arun Kumar, Bharti Sharma and Harsh K. Singh. Data analysis/interpretation was done by Harendra S. Bhoj and Pankaj Yadav. Manuscript drafting was done by Tapan K. Singha and Rajat Maheshwari. Statistical analysis was done by Harendra S. Bhoj and Pankaj Yadav. Critical revision of manuscript and admin, technical or material support was done by Sonal Mishra and Rajeshwar P. Sinha. Supervision and final approval were done by Rajeshwar P. Sinha.

6. FUNDING

This work was financially supported by Pathkits Healthcare Pvt. Ltd. and Molecular Quest Healthcare Pvt. Ltd.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

10. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

11. ACKNOWLEDGMENTS

The authors are highly thankful to Pathkits Healthcare Pvt. Ltd. for providing all the infrastructure and support to complete the work. We are also grateful to Molecular Quest Healthcare Pvt. Ltd for providing all the samples to carry out the research. Sonal Mishra is thankful to CSIR-UGC, New Delhi, India, for the financial support in the form of fellowship as Senior Research Fellow (Joint CSIR-UGC JRF-2019/ NTA Ref. No.: 191620046790).

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How to cite this article:
Singh S, Singha T, Maheshwari R, Yadav P, Kumar A, Bhoj H, Sharma B, Verma A, Gupta A, Singh H, Mishra S, Sinha R. Development of colloidal gold nanoparticle based lateral-flow assay for rapid detection of SARS-CoV-2 showing enhanced sensitivity and specificity. J App Biol Biotech. 2023;11(2):253-258.
DOI: 10.7324/JABB.2023.110228